

1     Claims

- 2
- 3     1.    A human embryonic stem cell line
- 4           characterised by at least one of the
- 5           following:
- 6           i)   presence of the cell surface markers TRA-
- 7                1-60, GTCM2, and SSEA-4;
- 8           ii)   expression of *Oct-4*;
- 9           iii)  expression of *NANOG*;
- 10          iv)  expression of *REX-1*; and/or
- 11               expression of *TERT*.
- 12
- 13    2.    The human stem cell line as claimed in Claim
- 14           1 having two or more of the characteristics
- 15           i) to v).
- 16
- 17    3.    The human stem cell line as claimed in Claim
- 18           2 having three or more of the characteristics
- 19           i) to v).
- 20
- 21    4.    The human stem cell line as claimed in Claim
- 22           3 having four of the characteristics i) to
- 23           v).
- 24
- 25    5.    The human stem cell line as claimed in Claim
- 26           4 having all of the characteristics i) to v).
- 27
- 28    6.    The stem cell line hES-NCL1 deposited at
- 29           NIBSC under Accession No. P-05-001.
- 30
- 31    7.    An embryonic stem cell bank comprising a
- 32           multiplicity of genetically distinct stem

1 cell lines as claimed in any one of Claims 1  
2 to 6.

3

4 8. A method of screening an agent for toxicity  
5 and/or for therapeutic efficacy, said method  
6 comprising:

- 7 i. exposing a stem cell line as claimed in  
8 any one of Claims 1 to 6 to said agent;  
9 ii. monitoring any alteration in viability  
10 and/or metabolism of said stem cells; and  
11 iii. determining any toxic or therapeutic  
12 effect of said agent.

13

14 9. A method of screening an agent for toxicity  
15 and/or for therapeutic efficacy, said method  
16 comprising:

- 17 i. exposing an embryonic stem cell bank as  
18 claimed in Claim 7 to said agent;  
19 ii. monitoring any alteration in viability  
20 and/or metabolism of said stem cells;  
21 and  
22 iii. determining any toxic or therapeutic  
23 effect of said agent.

24

25 10. A method of producing fibroblast-like cells,  
26 said method comprising:

- 27 i. providing a stem cell line as claimed in  
28 any one of Claims 1 to 6;  
29 ii. allowing cells of said stem cell line to  
30 differentiate into stem cell derived  
31 fibroblast-like cells.

32

- 1 11. The method of Claim 10 which is conducted  
2 without use of a specific stimulant for  
3 differentiation.  
4
- 5 12. The method as claimed in either one of Claims  
6 10 and 11 wherein the fibroblast-like cells  
7 are produced for a therapeutic purpose.  
8
- 9 13. A method of culturing cells wherein the  
10 fibroblast-like cells obtained as claimed in  
11 Claims 10 or 11 act as feeder cells or  
12 condition cell culture media used during  
13 culture of the cells.  
14
- 15 14. The method as claimed in Claim 13 wherein the  
16 cells being cultured are stem cells.  
17
- 18 15. A method of maintaining the viability of eggs  
19 prior to or during fertilisation, wherein the  
20 fibroblast-like cells obtained as claimed in  
21 Claims 10 or 11 act as feeder cells or  
22 condition cell culture media used during  
23 maintenance of the eggs.  
24
- 25 16. A method of culturing a blastocyst or embryo  
26 prior to implantation into a receptive  
27 female, wherein the fibroblast-like cells  
28 obtained as claimed in Claims 10 or 11 act as  
29 feeder cells or condition cell culture media  
30 used during culture of the blastocyst or  
31 embryo.  
32

- 1     17.     The fibroblast-like cell line hESCdF-NCL as  
2             deposited at ECACC under Accession No.  
3             04010601.  
4
- 5     18.     A method of culturing cells wherein hESCdF-  
6             NCL cells act as feeder cells or condition  
7             cell culture media used during culture of the  
8             cells.  
9
- 10    19.     The method as claimed in Claim 18 wherein the  
11             cells being cultured are stem cells.  
12
- 13    20.     A method of maintaining the viability of eggs  
14             prior to or during fertilisation, wherein  
15             hESCdF-NCL cells act as feeder cells or  
16             condition cell culture media used during  
17             maintenance of the eggs.  
18
- 19    21.     A method of culturing a blastocyst or embryo  
20             prior to implantation into a receptive  
21             female, wherein hESCdF-NCL cells act as  
22             feeder cells or condition cell culture media  
23             used during culture of the blastocyst or  
24             embryo.  
25
- 26    22.     A self-feeder system for the growth of  
27             undifferentiated stem cells, said system  
28             comprising:  
29             i.     culturing a stem cell line as claimed in  
30                   any one of Claims 1 to 6; and  
31             ii.    and allowing some of the cells of said  
32                   stem cell line to differentiate into

1 stem cell derived fibroblast-like cells  
2 whilst the remainder of the cells of  
3 said embryonic stem cell line remain in  
4 an undifferentiated pluripotent,  
5 multipotent or unipotent state, whereby  
6 said stem cell derived fibroblast-like  
7 cells act as autogeneic feeder cells for  
8 said stem cells.  
9

- 10 23. A method of culturing a blastocyst, said  
11 method comprising exposing said blastocyst  
12 for a period of at least 12 hours to Buffalo  
13 rat liver cells or to media conditioned by  
14 Buffalo rat liver cells.  
15
- 16 24. The method as claimed in Claim 23 wherein the  
17 period of exposure is at least 48 hours.  
18
- 19 25. The method as claimed in either one of Claims  
20 23 and 24 wherein the period of exposure of  
21 said blastocyst to said Buffalo rat liver  
22 cells or to media conditioned by said Buffalo  
23 rat liver cells immediately precedes  
24 extraction of ICM cells from the blastocyst.  
25
- 26 26. The method as claimed in any one of Claims 23  
27 to 25 wherein the media conditioned by  
28 Buffalo rat liver cells is produced by:  
29 i. culturing at least 75000 Buffalo rat  
30 liver cells/cm<sup>2</sup> in Glasgow medium for 24  
31 to 36 hours; and

1           ii.    recovering the media by removal of the  
2                   cells.

3

4    27.    The method as claimed in any one of Claims 23  
5           to 26 wherein the blastocyst can be cultured  
6           to day 8 after fertilisation and retain  
7           pluripotency.

8

9    28.    The method as claimed in any one of Claims 23  
10           to 27 wherein said blastocyst is a primate  
11           blastocyst.

12

13   29.    The method as claimed in Claim 28 wherein  
14           said blastocyst is a human blastocyst.

15

16   30.    A method for culturing a blastocyst, as  
17           claimed in any one of Claims 23 to 29, said  
18           method comprising:

- 19           i.       culturing said blastocyst from  
20                   fertilisation in G1 media;  
21           ii.       transferring said blastocyst of step  
22                   i) to G2.3 media and maintaining said  
23                   blastocyst in the G2.3 media; and  
24           iii.       transferring said blastocyst of step  
25                   ii) to cell culture media conditioned  
26                   by Buffalo rat liver cells.

27

28   31.    The method as claimed in Claim 30 wherein the  
29           blastocyst is cultured in the conditions of  
30           step i. for 1 to 3 days.

31

- 1     32.     The method as claimed in either one of Claims  
2             30 and 31 wherein the blastocyst is cultured  
3             in the conditions of step ii. for 2 to 3  
4             days.  
5
- 6     33.     The method as claimed in any one of Claims 30  
7             to 32 wherein the blastocyst is cultured in  
8             the conditions of step iii. for 1 to 3 days.  
9
- 10    34.     The method as claimed in any one of Claims 30  
11             to 33 wherein the cell culture media is  
12             Dulbecco's modified Eagle's medium optionally  
13             supplemented with 15% (v/v) Glasgow medium  
14             and conditioned by Buffalo rat liver cells.  
15
- 16    35.     A method of *in vitro* fertilisation, said  
17             method comprising culturing a blastocyst as  
18             claimed in any one of Claims 23 to 34; and  
19             implanting said cultured blastocyst into a  
20             receptive female.  
21
- 22    36.     A method of producing an embryonic stem cell  
23             line, said method comprising:  
24             i.     culturing a blastocyst as claimed in any  
25                     one of Claims 23 to 34; and  
26             ii.    extracting cells of the inner cell mass  
27                     (ICM) from said blastocyst and culturing  
28                     the cells to produce an embryonic stem  
29                     cell line therefrom.  
30

1     37.     The method as claimed in Claim 36 wherein  
2     said stem cell line is a primate embryonic  
3     stem cell line.

5     38.     The method as claimed in Claim 37 wherein  
6             said stem cell line is a non-human primate  
7             embryonic stem cell line.

9        39.        The method as claimed in Claim 37 wherein  
10                said stem cell line is a human embryonic stem  
11                cell line.

13     40.     The method as claimed in any one of Claims 36  
14             to 38 wherein said embryonic stem cell line  
15             is a pluripotent stem cell line.

17 41. A self-feeder system for the growth of  
18 undifferentiated stem cells, said system  
19 comprising:  
20 i. culturing a blastocyst as claimed in  
21 Claims 23 to 34;  
22 ii. extracting cells of the ICM from said  
23 blastocyst and culturing the cells to  
24 produce an embryonic stem cell line  
25 therefrom; and  
26 iii. and allowing some of the cells of said  
27 embryonic stem cell line to differentiate  
28 into stem cell derived fibroblast-like  
29 cells whilst the remainder of the cells  
30 of said embryonic stem cell line remain  
31 in an undifferentiated pluripotent,  
32 multipotent or unipotent state, whereby



1           said stem cell derived fibroblast-like  
2           cells act as autogeneic feeder cells for  
3           said stem cells.

4

5    42.    An embryonic stem cell bank comprising a  
6           multiplicity of genetically distinct stem  
7           cell lines obtained by the method as claimed  
8           in any one of Claims 36 to 39.

9

10   43.    A method of producing fibroblast-like cells,  
11           said method comprising:

12           i.    culturing a blastocyst as claimed in any  
13           one of Claims 23 to 34;

14           ii.   extracting cells of the ICM from said  
15           blastocyst and culturing the cells to  
16           produce an embryonic stem cell line  
17           therefrom; and

18           iii.   allowing cells of said embryonic stem  
19           cell line to differentiate into stem cell  
20           derived fibroblast-like cells.

21

22   44.    A method of culturing cells wherein the  
23           fibroblast-like cells obtained by the method  
24           of Claim 43 act as feeder cells or condition  
25           cell culture media used during culture of the  
26           cells.

27

28   45.    A method of maintaining the viability of eggs  
29           prior to or during fertilisation wherein the  
30           fibroblast-like cells obtained by the method  
31           of Claim 43 act as feeder cells or condition

1 cell culture media used during maintenance of  
2 the eggs.

3

4 46. A method of a blastocyst or embryo prior to  
5 implantation into a receptive female wherein  
6 the fibroblast-like cells obtained by the  
7 method of Claim 43 act as feeder cells or  
8 condition cell culture media used during  
9 culture of blastocyst or embryo.  
10